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BIOAVAILABILITY OF CYANIDE AND METAL–CYANIDE MIXTURES TO AQUATIC LIFE

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Abstract—Cyanide can be toxic to aquatic organisms, and the U.S. Environmental Protection Agency has developed ambient water-quality criteria to protect aquatic life. Recent work suggests that considering free, rather than total, cyanide provides a more accurate measure of the biological effects of cyanides and provides a basis for water-quality criteria. Aquatic organisms are sensitive to free cyanide, although certain metals can form stable complexes and reduce the amount of free cyanide. As a result, total cyanide is less toxic when complexing metals are present. Cyanide is often present in complex effluents, which requires understanding how other components within these complex effluents can affect cyanide speciation and bioavailability. The authors have developed a model to predict the aqueous speciation of cyanide and have shown that this model can predict the toxicity of metal–cyanide complexes in terms of free cyanide in solutions with varying water chemistry. Toxicity endpoints based on total cyanide ranged over several orders of magnitude for various metal–cyanide mixtures. However, predicted free cyanide concentrations among these same tests described the observed toxicity data to within a factor of 2. Aquatic toxicity can be well-described using free cyanide, and under certain conditions the toxicity was jointly described by free cyanide and elevated levels of bioavailable metals. Environ. Toxicol. Chem. 2012;31:1774–1780. © 2012 SETAC

Keywords—Water-quality criteria Mixture Cyanide Bioavailability Biotic ligand model

INTRODUCTION

Cyanide is an important industrial chemical in the metal-working and mining industries, along with other industrial applications, but it can also form in industrial and municipal wastewaters as a by-product following disinfected by chlorination [1]. Total cyanide will typically consist of numerous forms that include free cyanide and various metal–cyanide complexes. Free cyanide is highly toxic [2–12] and the most bioavailable of the expected cyanide species. As a result, toxicity can be moderated through complexation with trace metals [2,5,6,13].

For example, Doudoroff [5] and Doudoroff et al. [6] demonstrated that various divalent metal–cyanide complexes resulted in observed mortality that spanned nearly four orders of magnitude in terms of total cyanide and varied with the type of metal and specific water chemistry (e.g., pH, alkalinity) during the exposures. Pablo et al. [7–10] performed toxicity tests with ferric and ferrous iron–cyanide solutions as well as sodium cyanide for several marine organisms. On a total cyanide basis, the presence of iron–cyanide solutions increased the observed toxicity by up to an order of magnitude above the toxicity observed for simple cyanide salts. Broderius [13] reported similar results for exposure of fish to nickel and silver cyanide solutions.

The present study evaluates the bioavailability of cyanide to aquatic organisms using a chemical equilibrium model and provides validation using published toxicity studies. The model was used to support the reevaluation of the U.S. Environmental Protection Agency's (U.S. EPA) cyanide criteria based on free cyanide [11] by evaluating toxicity tests with metal–cyanide mixtures. The present study corroborates the results from earlier studies that show that toxicity in metal–cyanide mixtures is primarily due to free cyanide [5,6,13]. In addition, we observed that under certain conditions, metals in these same mixtures can exhibit some toxicity, and the degree to which metals may be toxic was estimated using the biotic ligand model (BLM) by quantifying the accumulation of metal on the biotic ligand sites in sensitive aquatic organisms. The BLM was also used as the speciation model for cyanide, thereby providing information on the bioavailability of both cyanide and metal mixtures in a single calculation.

METHODS

Toxicity data

Toxicity data were compiled from the peer-reviewed literature for aquatic organisms exposed to cyanide–metal mixtures. Tests were screened for those that reported measured concentrations of free cyanide and exposure in water chemistry (e.g., pH, Ca, Mg, SO_4, Cl, dissolved organic carbon [DOC], total cyanide, total metal). For simulations with marine water, the only information provided was the salinity. In these cases, the BLM input parameters were estimated using typical seawater chemistry [14]. This assumption is not expected to introduce appreciable error into the modeling results because the major ions in seawater have a weak interaction with cyanide compared to the metal–cyanide complexes in those exposures. Measured total concentrations of metals and cyanide are preferred inputs to the model. However, the data compiled for use in the present study reported nominal total metal and total cyanide concentrations, which were used as inputs to the speciation model. A trace amount of DOC (e.g., 0.5 mg/L) was assumed to be present in tests where synthetic waters were used with no added DOC. This assumption is consistent with other metal toxicity tests used to develop the BLM for metals [15–17] and reflects small quantities of organic matter that are expected in exposures involving aquatic organisms, even when no external

All Supplemental Data may be found in the online version of this article.

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Table 1. Summary of toxicity studies used in the present study

Common name	Organism	Metal–cyanide mixture	Water chemistry	Endpoint	Source
Australian bass	*Macquaria novemaculeata*	Fe(III), Fe(II), NaCN	Seawater: pH, temperature, salinity	Survival at 96 h	[7]
Black bream	*Acanthopagrus butcheri*	Fe(III), Fe(II), NaCN	Seawater: pH, temperature, salinity	Survival at 96 h	[7]
Banana prawn	*Penaeus monodon*	Fe(III), Fe(II), NaCN	Seawater: pH, temperature, salinity	Survival at 96 h	[9]
Marine diatom	*Nitzschia closterium*	Fe(III), Fe(II), NaCN	Seawater: pH, temperature, salinity	Survival at 96 h	[10]
Doughboy scallop larvae	*Chlamys asperrimus*	Fe(III), Fe(II), NaCN	Seawater: pH, temperature, salinity	Survival at 96 h	[8]
Fathead minnow juveniles	*Pimephales promelas*	Cd, Cu(II), Ni, Zn, NaCN	Variable water quality: pH, trace and major ion content	Survival at 96 h	[5]
Bluegill juveniles	*Lepomis macrochirus*	Ag, Cd, Cu(II), Ni, Zn, NaCN	Variable water quality: pH, trace and major ion content	MST	[6]
Stickleback juveniles	*Gasterosteus aculeatus*	Ag, Ni, NaCN	Variable water quality: pH, trace and major ion content	MST	[13]

MST = median survival time.

source of DOC is present. The data sets used for the present study included dose–response data for cyanide exposures as well as median survival time (MST) tests for metal–cyanide mixtures including nickel, zinc, copper, cadmium, and silver (Table 1). Photo-redox reactions in ferrous cyanide mixtures are usually kinetically controlled [18]; therefore, they are not amenable to the present analysis using a chemical equilibrium model and were subsequently excluded.

Speciation model

The chemical equilibrium model was developed by evaluating stability constants for metal–cyanide complexes for internal consistency and for performance against measured free cyanide in the toxicity tests identified above. Two main sources of the stability constants were used to develop the speciation model (Table 2). The U.S. EPA's MINTEQ database [19] was the primary source of the Fe(III) cyanide reactions and is deemed more reliable than other sources [20]. For other metal–cyanide reactions (e.g., Ag, Cd, Cu, Ni, Zn), the National Institute of Standards and Technology database of critically reviewed stability constants [21,22] was used to consider the effects of metals on cyanide speciation in metal–cyanide mixtures (Table 1) reported in the present study. Model performance for nickel–cyanide solutions was improved by adding two reactions forming $Ni(CN)_2$ and $Ni(CN)_3$ from the MINTEQ database (Table 2) [19]. Copper–cyanide solutions were simulated as Cu(II) based on the reported exposure conditions and performance of the model relative to the data.

In some tests, nickel carbonate and silver cyanide solubility was important and modeled using $NiCO_{3(s)}$ and $AgCN_{(s)}$ from the National Institute of Standards and Technology database. Speciation calculations were run using the speciation model CHESS [23], which is the computational engine of the BLM [15,24]. Incorporating the cyanide reactions and bioavailability calculations within the BLM also provided a means to evaluate potential cotoxicity of metals via simulating metal accumulation on the biotic ligand, such as the gill or another sensitive biological interface that contains proteins or other biomolecules with ligands that can bind metals. A standard output of the BLM is predicting the amount of metal accumulated on the

Table 2. Metal–cyanide reactions used in speciation model

Species and formation reactions									Log_K	Source
Ag^+	+	$2CN^-$			=	$Ag(CN)^-$			20.48	[21]
Ag^+	+	$3CN^-$			=	$Ag(CN)_3^{-2}$			21.70	[21]
Ag^+	+	H_2O	+	CN^-	=	$Ag(OH)(CN)^-$	+	H^+	−0.78	[21]
Cd^{+2}	+	$2CN^-$			=	$Cd(CN)_2$			11.12	[21]
Cd^{+2}	+	$3CN^-$			=	$Cd(CN)_3^-$			15.65	[21]
Cd^{+2}	+	$4CN^-$			=	$Cd(CN)_4^{-2}$			17.92	[21]
Cd^{+2}	+	CN^-			=	$Cd(CN)^+$			6.01	[21]
Cu^{+2}	+	$4CN^-$			=	$Cu(CN)_4^{-2}$			28.50	[24]
H^+	+	CN^-			=	HCN			9.21	[21]
Ni^{+2}	+	$2CN^-$			=	$Ni(CN)_2^{-2}$			14.59	[19]
Ni^{+2}	+	$3CN^-$			=	$Ni(CN)_3^{-2}$			22.63	[19]
Ni^{+2}	+	$4CN^-$			=	$Ni(CN)_4^{-2}$			30.20	[21]
Ni^{+2}	+	H^+	+	$4CN^-$	=	$NiH(CN)_4^-$			35.60	[21]
Ni^{+2}	+	$2H^+$	+	$4CN^-$	=	$NiH_2(CN)_4$			40.10	[21]
Ni^{+2}	+	$3H^+$	+	$4CN^-$	=	$NiH_3(CN)_4^+$			42.70	[21]
Zn^{+2}	+	$2CN^-$			=	$Zn(CN)_2$			11.07	[21]
Zn^{+2}	+	$3CN^-$			=	$Zn(CN)_3^-$			16.05	[21]
Zn^{+2}	+	$4CN^-$			=	$Zn(CN)_4^{-2}$			19.62	[21]
Fe^{+3}	+	Ca^{+2}	+	$6CN^-$	=	$CaFe(CN)_6^-$			55.47	[19]
$2Fe^{+3}$	+	$6CN^-$			=	$Fe_2(CN)_6$			56.98	[19]
Fe^{+3}	+	$6CN^-$			=	$Fe(CN)_6^{-3}$			52.63	[19]
Fe^{+3}	+	K^+	+	$6CN^-$	=	$KFe(CN)_6^{-2}$			54.07	[19]
Fe^{+3}	+	Mg^{+2}	+	$6CN^-$	=	$MgFe(CN)_6^-$			55.39	[19]
$AgCN_{(s)}$					=	Ag^+	+	CN^-	−11.20	[19]
$NiCO_{3(s)}$					=	Ni^{+2}	+	CO_3^{-2}	−15.74	[19]

biotic ligand, which is then compared to the species-specific median lethal accumulation level for an estimate of the potential for cotoxicity of Ni and Ag in these metal–cyanide mixture exposures.

RESULTS

Speciation model

The speciation model was validated by comparing predicted free cyanide to measured free cyanide concentrations (e.g., hydrogen cyanide [HCN], CN^-) in various metal–cyanide mixtures (Fig. 1). These speciation data are from measured free cyanide concentrations that occurred in toxicity tests (Table 1), as well as measured concentrations that resulted from chemical equilibration studies [13] for Fe(III)–, Cu(II)–, Ag–, Cd–, Zn–, and Ni–cyanide solutions. Approximately 80% of the measurements are within a factor of 2 of the model simulations, and 90% are within a factor of 5. The variability between the model and measurements is consistent with other chemical equilibrium-based toxicity models (e.g., BLM), which have been used previously for assessing hazard and developing water-quality criteria [15,24]. This version of the BLM, therefore, is considered suitable for evaluating cyanide bioavailability.

As noted, tests with Fe(II) were not well-described with this chemical equilibrium model due to photodecomposition and oxidation of those complexes. The model could, however, describe cyanide complexation with ferric iron, Fe(III), except when those solutions were irradiated. Total and free cyanide concentrations in Fe(III)–cyanide exposures appear to be generally stable under typical experimental conditions for fish and invertebrates (Table 1) [7–9] and agree with the predictions within a factor of 2. In a few tests with Fe(III)–cyanide, the measured HCN [10] is substantially greater than the model predictions (Fig. 1, open triangles). These are from cyanide exposures to marine diatoms that were subject to a higher degree of illumination (~13 klux, fluorescent) than other tests (<1.1 klux, fluorescent), which likely caused photodecomposition of the initial Fe(III)–cyanide complexes and elevated release of free cyanide.

The model predictions of the toxicity of the Cu(II) complexes slightly underpredict measured toxicity. It is possible that some portion of the Cu(II) is reduced to Cu(I) by cyanide [25], thereby leading to Cu(I)–cyanide complexes, which are substantially more stable than Cu(II) complexes (Table 2). The stronger binding of Cu(I) complexes would result in lower concentrations of free cyanide in solution [19,21,22] and lower observed toxicity.

Dose–response analysis

Metal–cyanide toxicity to fathead minnow (*Pimephales promelas*), expressed on a total cyanide basis (Fig. 2A), spans over four orders of magnitude depending on the metal used in the tests and the chemistry of the exposure water (e.g., pH, alkalinity). The model-predicted free cyanide concentrations (Fig. 2B) are in much better agreement with each other and are consistent with the observed toxicity in sodium cyanide tests. The estimated median lethal concentration (LC50) for fathead minnow exposed to sodium cyanide is 212 μg/L. The LC50s for the metal–cyanide mixtures range from 146 to 288 μg/L (excluding the Cu–cyanide prediction, based on low confidence in that model; see *Speciation model*) based on predicted free cyanide. This is consistent with the species mean acute value of 125 μg/L (range, 120–272 μg/L) [11]. The variability in the

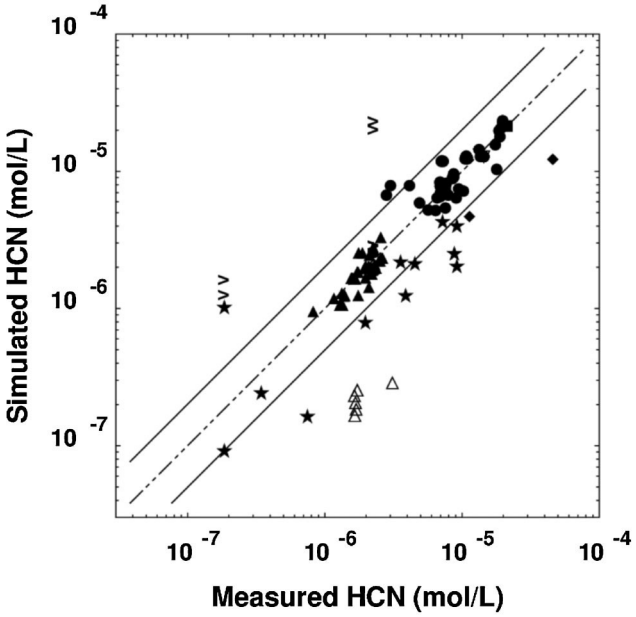

Fig. 1. Comparison of measured and simulated free cyanide in various metal–cyanide mixtures of Fe(III) (▲), irradiated Fe(III) tests (Δ), Ag (★), Cd (◆), Cu(II) (■), Zn (▽), and Ni (●) (88% within two times, $n = 117$, excluding those with suspect analytical). > = studies with reported concentrations where the samples were affected by volatilization and were not considered representative of actual exposure conditions, which were expected to be greater than the reported concentrations [7,9,13].

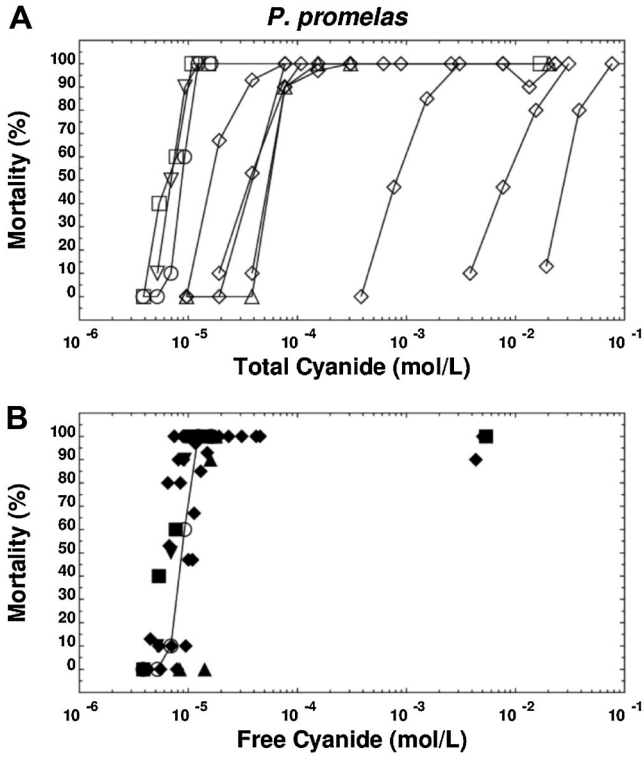

Fig. 2. Comparison of mortality with total cyanide (**A**) and free cyanide (**B**) to *Pimephales promelas* for individual series of metal–cyanide mixtures (Δ = Cu[II]; ▽ = Zn; □ = Cd; ◇ = Ni) and sodium cyanide solutions (○), including reported total cyanide (open symbols) and simulated free cyanide concentrations (filled symbols).

relationship between the simulated free cyanide toxicity and toxicity is relatively low (e.g., ± factor of 2) and is consistent with the observed variability in other metal exposures to fish [13]; therefore, we conclude that free cyanide is the primary toxic agent in these exposures based on the chemical equilibrium simulations. The dose–response for the various metal–cyanide and sodium cyanide tests are typically very steep where the difference between low mortality (e.g., <20% mortality) is within a factor of 2 of near complete mortality (e.g., >80% mortality).

Additional validation data sets are presented in Figures 3 to 6 for a series of marine organisms [7–9] and provide additional lines of evidence from independent studies with different organisms and exposure conditions. As noted, the model performance for ferric iron–cyanide complexes is generally very good (Fig. 1). Observed mortality of the Australian sea bass (*Macquaria novemaculeata*) on a total cyanide basis is approximately two orders of magnitude greater than the corresponding sodium cyanide exposures (Fig. 3A). However, the dose–response curve for Fe(III)–cyanide exposure is within a factor of 2 of the analogous sodium cyanide exposures (Fig. 3B). The approximate LC50 of the sodium cyanide test was 102 μg/L (95% confidence interval 102–107), and the LC50 from the Fe–cyanide test was >51 μg/L based on predicted free cyanide. The observed dose response for this organism in the Fe–cyanide test is very steep but incomplete because a few exposures resulted in partial mortalities in these tests. Also, simulated free cyanide concentrations are qualitatively consistent with the measured free cyanide concentrations, even though many of the measurements are reported as "greater-than" concentrations due to confounded analytical results [7].

A similar comparison can be made for Fe(III)–cyanide exposures to the black bream, *Acanthopagrus butcheri* [7]

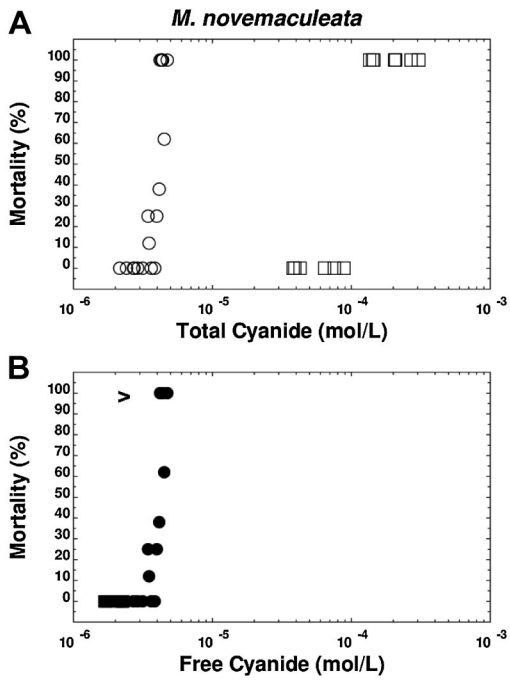

Fig. 3. Comparison of mortality with total cyanide (**A**) and free cyanide (**B**) to *Macquaria novemaculeata* exposures to ferric cyanide including measured total cyanide (□), predicted free cyanide in ferric cyanide solutions (■), sodium cyanide solutions (○), and predicted free cyanide in sodium cyanide solutions (●). > = reported values and represent measurements where the actual concentrations are expected to be greater than the reported measurements [7].

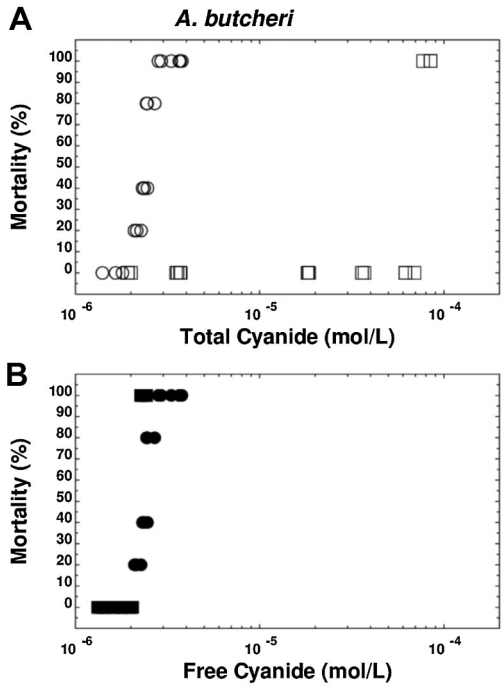

Fig. 4. Comparison of mortality with total cyanide (**A**) and free cyanide (**B**) to *Acanthopagrus butcherii* exposures to ferric cyanide including measured total cyanide (□), predicted free cyanide in ferric cyanide solutions (■), sodium cyanide solutions (○), and predicted free cyanide in sodium cyanide solutions (●).

(Fig. 4). On a total cyanide basis (Fig. 4A) the dose response for the Fe(III)–cyanide exposure is offset by approximately a factor of 10 from the sodium cyanide tests. In contrast, the dose response based on the simulated and measured free cyanide concentrations (Fig. 4B) agrees very well with the sodium cyanide tests. The observed dose responses are very steep, which is consistent with other species discussed above. The LC50 is 62 (CI 60–64) μg/L for sodium cyanide and 53 (CI 34–73) μg/L for the Fe–cyanide test based on predicted free cyanide. A few exposures in this series of tests generated partial mortalities, which resulted in a very steep observed dose response that was slightly more pronounced than the dose response from the analogous sodium cyanide tests. This, however, is consistent with the variability for free cyanide toxicity tests [13] and consistent with the hypothesis that free cyanide is the primary toxic agent in these exposures.

Tests with a marine bivalve, the scallop *Chlamys asperrimus*, showed a similar comparison [8] (Fig. 5). The observed dose response on a total cyanide basis (Fig. 5A) varies by an order of magnitude between the Fe(III)–cyanide and sodium cyanide tests. Very good agreement exists, however, between the dose response based on the measured and simulated free cyanide in the Fe(III)–cyanide exposure and the sodium cyanide dose response (Fig. 5B and Supplementary Data, Table S1). The model-simulated free cyanide concentrations have a similar dose response and agree well with the dose responses for the sodium cyanide and Fe(III)–cyanide tests on a free cyanide basis (Supplementary Data, Table S1).

Additional tests on the marine prawn *Penaeus monodon* show differences of up to two orders of magnitude between the sodium cyanide and Fe(III)–cyanide tests on a total cyanide basis [9] (Fig. 6A). The dose responses on a free cyanide basis for both measured and simulated (Fig. 6B) concentrations,

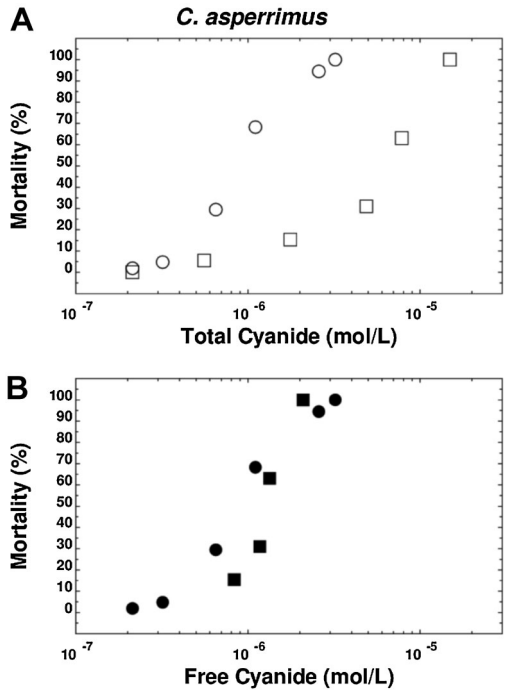

Fig. 5. Comparison of mortality with total cyanide (**A**) and free cyanide (**B**) to *Chlamys asperrimus* exposures to ferric cyanide including measured total cyanide (□), predicted free cyanide in ferric cyanide solutions (■), sodium cyanide solutions (○), and predicted free cyanide in sodium cyanide solutions (●).

however, agree very well with the sodium cyanide tests. (See Supplemental Data, Table S1 for LC50 and statistical comparisons.) The model simulations provided a consistent description of the observed mortality in terms of free cyanide from a series of exposure conditions with varying pH and cyanide and Fe(III) concentrations that resulted in a highly variable dose response on a total cyanide basis where exposure concentrations spanned nearly a factor of 10 at a specific mortality level (e.g., 40%).

Some variability is observed in free cyanide measurements at the upper end of the dose response (Fig. 6B), where concentrations were reported as "greater than the detection limit" due to analytical variability [9]. These data are plotted at the reported concentration as a ">" symbol to indicate that the actual concentrations are likely greater than the reported value. This is consistent with the corresponding model predictions, which, in this instance, are approximately an order of magnitude greater than reported placeholder values. Even with this qualitative comparison, it is clear that free cyanide is the primary toxic agent in these tests.

Observed mortality in the studies discussed above can vary over four orders of magnitude on a total cyanide basis in metal–cyanide exposures. This is due to the strong complexation of cyanide with Fe(III) and Ni and through mild complexation by Zn, Cd, and Cu(II). The dose responses based on predicted free cyanide in these metal-treated exposures were consistent with the analogous sodium cyanide tests (Supplementary Data, Table S1). These results strongly support the hypothesis that free cyanide is the primary toxic agent in metal–cyanide solutions. The model was able to respond to a wide range of water chemistries (e.g., pH, variable alkalinity in freshwaters, seawater, total metal, and cyanide concentrations; Table 1), to provide a reasonably consistent description of the available toxicity tests in terms of free cyanide.

Median survival time

Some of the available data sets that were useful demonstrations of cyanide bioavailability relationships characterized cyanide toxicity using MST but did not include corresponding LC50s. In these tests, Doudoroff et al. [6] and Broderius [13] exposed stickleback and bluegill to solutions of Ag– and Ni–cyanide with variable water chemistry for up to 24 h and reported MSTs. Total cyanide can be a poor predictor of MST in the presence of complexing metals. Furthermore, total cyanide concentration at the MST can vary substantially (e.g., >10 times) even for similar MST responses (Fig. 7A, D, G). This is due to the complexation behavior of Ni and Ag with cyanide, which is affected by varying water chemistry (e.g., pH, alkalinity). In addition, the concentration response curves are approximately one or two orders of magnitude greater than the analogous sodium cyanide exposures.

The metal–cyanide exposures compare favorably with sodium cyanide exposures when evaluated on a free cyanide basis (Fig. 7B, E, H). Generally, there is agreement between the simulated and measured free cyanide in the sodium cyanide and metal–cyanide exposures, which strongly supports the hypothesis that free cyanide is the primary toxic agent in metal–cyanide exposures. The MST levels off around 300 min for both the stickleback and bluegill exposures, showing only a slight decrease with increasing free cyanide, consistent with the steep dose responses in the exposures discussed above (Figs. 2–6).

Despite the very consistent description of the toxicity data using measured or simulated free cyanide concentrations, there are slight deviations from this relationship for the Ni–cyanide exposure to stickleback (Fig. 7B) that have MST between 100 and 300 min. In this region-free cyanide, concentrations are

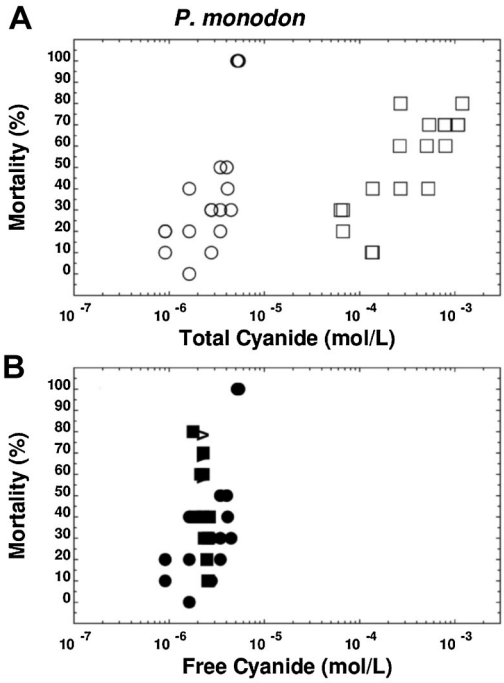

Fig. 6. Comparison of mortality with total cyanide (**A**) and free cyanide (**B**) to *Penaeus monodon* exposures to ferric cyanide including measured total cyanide (□), predicted free cyanide in ferric cyanide solutions (■), sodium cyanide solutions (○), and predicted free cyanide in sodium cyanide solutions (●). > = reported values and represent measurements where the actual concentrations are expected to be greater than the reported measurements [9].

relatively constant, yet the MST varies from approximately 100 to 300 min, somewhat in contrast to the sodium cyanide MST response curve. It is noted that this is a minor discrepancy and could be attributed to experimental variability, but further analysis shows that this observation is coincident with a relatively elevated amount of nickel bound to the biotic ligand estimated using the BLM (e.g., gill, Fig. 7C, lower portion). This was estimated using the fathead minnow BLM for nickel as a surrogate [26], because there are no Ni BLMs for stickleback and bluegill. The corresponding gill Ni concentrations are near the critical accumulation level (e.g., 3.3 nmol/gw [26]) used to predict acute LC50s for juvenile fathead minnows, suggesting that nickel under these exposure conditions may be contributing to the observed toxicity. The nature of this data set allows for only a semiquantitative evaluation of the cotoxicity of metals in these exposures.

Similar discrepancies are observed in the Ag–cyanide exposures (Fig. 7H), where free cyanide concentrations at MST of 700 min and longer decline sharply to below 1 μM (~26 μg/L cyanide). It is plausible that these free cyanide concentrations might be insufficient to cause the observed toxicity given the steep dose–response of acute toxicity in aquatic organisms exposed to free cyanide relative to the expected effect levels, as noted above [11]. However, the MST (~750–1,500) for this subset of the Ag–cyanide test series suggests additional toxic agents in this exposure. Simulated gill Ag accumulation was modeled with the BLM for fathead minnow [27]. Gill Ag concentrations are quite elevated in this range of MST (~700 min) and, in fact, exceed or are near the median lethal accumulation trigger level associated with acute toxicity of Ag in fathead minnow (e.g., 8.9 nmol/gw) in a few instances (Fig. 7I). This strongly suggests the cotoxicity of Ag in these Ag–cyanide exposures.

DISCUSSION

This chemical speciation model seems suitable for use with ferric cyanide and most metal–cyanide solutions (e.g., Ag, Ni, Cu[II], Cd, Zn). Model performance for ferrous cyanide solutions was poor and attributed to photo-redox instability of the Fe(II)–cyanide complexes. In addition, Doudoroff [5] noted that formation of iron–cyanide complexes using freshly prepared solutions of Fe(II)–sulfate and sodium cyanide salts is kinetically limited and could influence the applicability of this model under certain environmental conditions. Caution should be used when applying this model to field settings or to other laboratory data where the complexation reactions are kinetically limited or where photolysis of the Fe–cyanide complexes is likely to occur.

The model performed well for Fe(III)–cyanide solutions prepared using ferricyanide salts. Predicted free cyanide (Fig. 1) and predicted EC50s based on predicted free cyanide for a range of aquatic organisms (Figs. 3–6) agreed well with measured values.

This modeling analysis supports the hypothesis that free cyanide is the primary toxic agent in metal–cyanide exposures. This is based on the consistent description of the available toxicity data using free cyanide concentrations for a range of metal–cyanide mixtures and NaCN tests. The model showed strong correlation between the observed toxicity and simulated free cyanide concentrations, which were consistent with measured free cyanide concentrations where available.

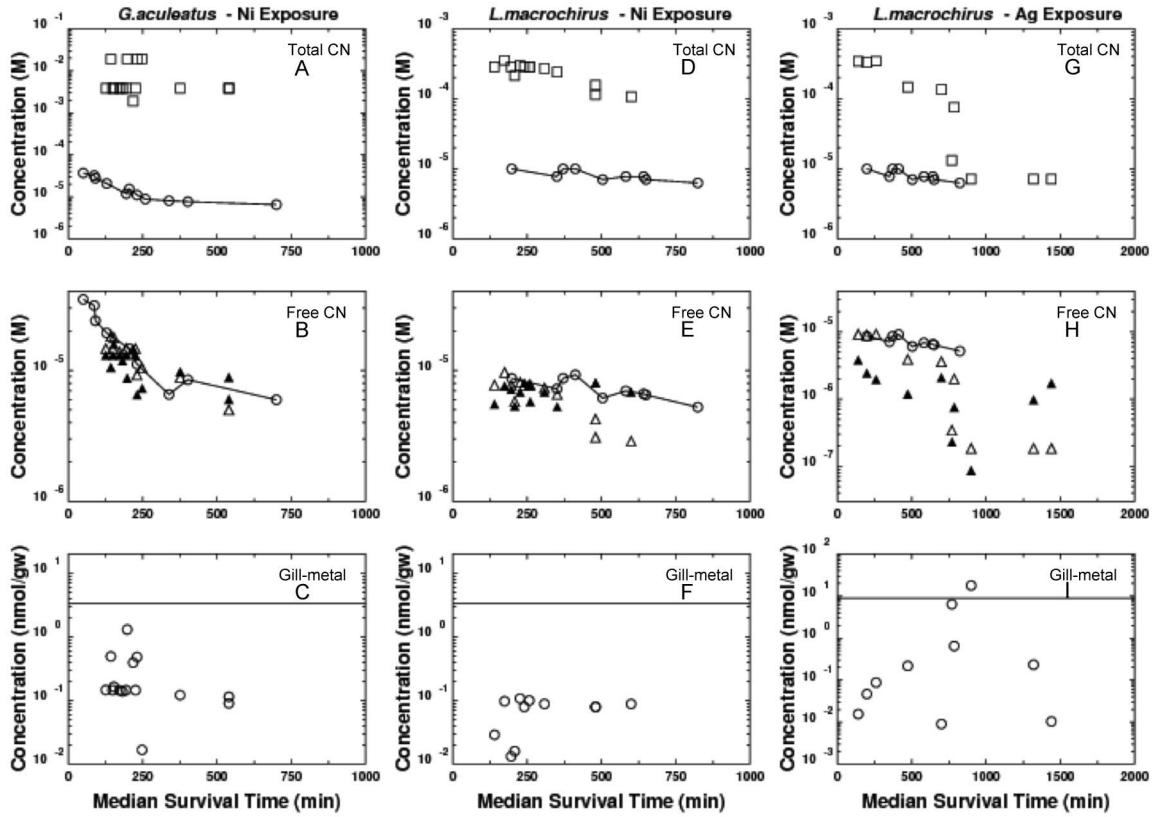

Fig. 7. Comparing median survival times (MSTs) in various sodium cyanide (○) and metal–cyanide (□) exposures (upper panels). The same MST responses on a free cyanide basis are in the middle set of panels from sodium cyanide (○) and metal–cyanide (Δ) exposures including model simulations (▲). Accumulation of nickel or silver on the biotic ligand (e.g., gill, ○) are given in the lower panels for stickleback (*Gasterosteus aculeatus*) and bluegill (*Lepomis macrochirus*). Note change in axis scales. Horizontal lines in **C**, **F**, and **I** represent the median lethal accumulation (e.g., LA50) of metal on the biotic ligand (e.g., gill).

Previous work [13] suggested that the metal–cyanide complexes themselves could be bioavailable, contributing to the overall toxic effect of the metal–cyanide mixture. However, there can be sufficient concentrations of bioavailable metals that result in accumulation of metal on the biotic ligand (e.g., gill) and can reach levels that appear to cause cotoxicity in metal–cyanide mixtures. The BLM and the cyanide speciation model discussed in the present study offer a readily applicable framework for evaluating the potential toxicity of both metals and cyanide to aquatic organisms.

SUPPLEMENTAL DATA

Table S1. Summary statistics of dose responses vs free cyanide.

Supplemental References. (57 KB DOC)

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REFERENCES

1. Kavanaugh M, Deeb R, Markowitz D, Dzombak D, Zheng A, Theis T, Young T, Luthy R. 2003. Cyanide formation and fate in complex effluents and its relation to water quality criteria. Report 98-HHE-5. Water Environment Research Foundation, Alexandria, VA, USA.
2. Eisler R, Clark DR Jr, Wiemeyer SN, Henny CJ. 1999. Sodium cyanide hazards to fish and other wildlife from gold mining operations. In Azcue JM, ed, *Environmental Impacts of Mining Activities: Emphasis on Mitigation and Remedial Measures*. Springer-Verlag, Berlin, Germany, pp 55–67.
3. U.S. Environmental Protection Agency. 1985. Ambient water quality criteria for cyanide— 1984. EPA 440/5-84/028. Technical Report. Washington, DC.
4. Gensemer R, DeForest D, Stenhouse A, Higgins C, Cardwell RD. 2006. Aquatic toxicity of cyanide. In Dzombak D, Ghosh R, Wong-Chong F, eds, *Cyanide in Water and Soil: Chemistry, Risk and Management*. Taylor and Francis/CRC Press, Boca Raton, FL, USA.
5. Doudoroff P. 1956. Some experiments on the toxicity of complex cyanides to fish. *Sewage Ind Waste* 28:1020–1040.
6. Doudoroff P, Leduc G, Schneider CR. 1966. Acute toxicity to fish of solutions containing complex metal cyanides in relation to concentrations of molecular hydrocyanic acid. *Trans Am Fish Soc* 95:6–22.
7. Pablo F, Buckney R, Lim R. 1996. Toxicity of cyanide and iron–cyanide complexes to Australian bass *Macquaria novemaculeata* and black bream *Acanthopagrus butcheri*. *Aust J Ecotoxicol* 2:75–84.
8. Pablo F, Buckney R, Lim RP. 1997. Toxicity of cyanide, iron–cyanide complexes and a blast furnace effluent to larvae of the doughboy scallop, *Chlamys asperrimus*. *Bull Environ Contam Toxicol* 58:93–100.
9. Pablo F, Buckney R, Lim RP. 1997. Toxicity of cyanide, iron–cyanide complexes and a blast-furnace effluent to the banana prawn, *Penaeus monodon*. *Bull Environ Contam Toxicol* 58:822–829.
10. Pablo F, Stauber J, Buckney RT. 1997. Toxicity of cyanide and cyanide complexes to the marine diatom *Nitzschia closterium*. *Water Res* 31:2435–2442.
11. Gensemer R, DeForest D, Cardwell R, Dzombak D, Santore R. 2007. Scientific review of cyanide ecotoxicity and evaluation of ambient water quality criteria. Project 01-ECO-1. Water Environment Research Foundation, Alexandria, VA, USA.
12. Brix K, Cardwell R, Henderson D, Marsden A. 2000. Site-specific marine water-quality criterion for cyanide. *Environ Toxicol Chem* 19: 2323–2327.
13. Broderius S. 1973. Determination of molecular hydrocyanic acid in water and studies of the chemistry and toxicity to fish of metal–cyanide complexes. PhD thesis. Oregon State University, Corvallis, OR, USA.
14. Stumm W, Morgan JJ. 1996. *Aquatic Chemistry. Chemical Equilibria and Rates in Natural Waters*. 3rd ed. John Wiley and Sons, New York, NY, USA.
15. Santore R, Di Toro D, Paquin P, Allen H, Meyer J. 2001. Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and *Daphnia*. *Environ Toxicol Chem* 20: 2397–2402.
16. U.S. Environmental Protection Agency. 2007. Update of ambient water quality criteria for copper. EPA 822/F-07/001. Technical Report. Washington, DC.
17. Santore RC, Mathew R, Paquin PR, Di Toro DM. 2002. Application of the biotic ligand model to predicting zinc toxicity to rainbow trout, fathead minnow, and *Daphnia magna*. *Comp Biochem Physiol, Part C: Toxicol Pharmacol* 133:271–285.
18. Meeussen JCL, Keizer MG, de Haan FAM. 1992. Chemical stability and decomposition rate of iron cyanide complexes in soil solutions. *Environ Sci Technol* 26:511–516.
19. Sehmel G. 1989. Cyanide and antimony thermodynamic database for the aqueous species and solids for the EPA-MINTEQ geochemical code. EPA PNL-6835, Pacific Northwest Laboratory, Richland, WA, USA.
20. Ghosh R, Dzombak D, Luthy R. 1999. Equilibrium precipitation and dissolution of iron cyanide solids in water. *Environ Eng Sci* 16:293–313.
21. Beck M. 1987. Critical survey of stability constants of cyano complexes. *Pure and Appl Chem* 59:1703–1720.
22. Smith R, Martell A, Motekaitis R. 2004. NIST Standard Reference Database 46. NIST Critically Selected Stability Constants of Metal Complexes Database v6. National Institute of Standards and Technolog, Gaithersburg, MD, USA
23. Santore R, Driscoll CT. 1995. The CHESS model for calculating chemical equilibria in soils and solutions. In Loeppert R, Schwab A, Goldberg S, eds, *Chemical Equilibrium and Reaction Models*. American Society of Agronomy, Madison, WI, USA.
24. Di Toro D, Allen H, Bergman H, Meyer J, Paquin R, Santore R. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ Toxicol Chem* 20:2383–2396.
25. Mudder T, Botz M, eds. 1998. *The Cyanide Monograph*, 2nd ed. Mining Journals Books, London, UK.
26. Wu B, Paquin P, Navab V, Mathew R, Santore R, Di Toro D. 2003. Development of a biotic ligand model for nickel: Phase I. Report 01-ECO-10-T, Water Environment Research Foundation, Alexandria, VA, USA.
27. Paquin P, Di Toro D. 2008. Silver biotic ligand model (BLM): Refinement of an acute BLM for silver. Report 99-ECO-1-2T, Water Environment Research Foundation, Alexandria, VA, USA.